Comments on ETBE and *tert*-Butanol (TBA) Preliminary Materials for IRIS Toxicological Review

General and Non-Cancer Endpoints

On Behalf of
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Tox Strategies

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General Comments
ETBE/TBA Preliminary Materials

- Draft literature strategy; processes for identifying and screening scientific literature and literature results
- Preliminary evidence tables, and
- Preliminary exposure-response arrays

Both summarizing key characteristics and findings from studies that EPA proposes to consider in identifying hazards and characterizing exposure response relationships.

The stated purpose of these materials is to document EPA's systematic review of the scientific literature, demonstrate a standardized approach to presenting key study data, and foster early public involvement and transparency.

General Comments
ETBE/TBA Preliminary Materials

Lyondell has reviewed the preliminary materials and offers for consideration our comments on:

- the clarity and transparency of the materials;
- the approach for identifying pertinent studies;
- the selection of studies for data extraction to preliminary evidence tables and exposure-response arrays;
- methodological considerations that could affect the interpretation of, or confidence in, study results; and
- additional studies published or nearing publication that may provide data for the evaluation of human health hazard or exposure-response relationships.

Draft Literature Search Strategy (ETBE/TBA)

- Well documented database search and strategy; > 600 citations for ETBE, > 2900 for TBA identified; European Chemical Agency (ECHAs) database for registered substances: http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances
- For complete clarity and transparency, further text is recommended to support the outline (Figure 1.1) of the literature search approach.
- Specific criteria for selection of primary vs. not primary sources of data; ***Request EPA provide their worksheet that details the basis for selecting and/or excluding citations.***
- Citations identified as "Not Primary Source of Health Effects Data"
  - What is the decision process for including specific citations?
  - Will they be reviewed for quality prior to incorporation?
  - Will there be an opportunity for public comment prior to finalizing?

Preliminary Evidence Tables (ETBE/TBA)

- Decision process for data extraction; ***unclear why certain endpoints are considered individually and others grouped in evidence tables and exposure response arrays.***
- Recommendation that study quality be considered prior to including data; ***consider similar approach used by OECD (Klimisch et al., 1997).***
- Request clarity on data normalization strategy used; ***although helps to make studies comparable, it is not the same as extracting the specific data.***
- Clarify why some reproductive endpoints were presented as incidence and endpoints as percentage change.
- Statistical analysis of data; ***Not clear what data was statistical analyzed.***

Preliminary Evidence Tables (ETBE/TBA)

Selection of Studies and Endpoints for Data Extraction

- Borghoff et al., (2001) was only considered for mechanism data and not other endpoints of toxicity because it was a 10-day study.
- Inconsistency between reviews for selected endpoints extracted; ***Serum enzymes for ETBE but not for TBA***.
- Certain studies were selected for body weight changes and other studies not used (section 2.6 of TBA review).
- The approach used does not allow for consideration of how changes in one endpoint influences a change in another; data selected in isolation.
  - Organ weight changes without histology
  - Body weight changes without food and water consumption
  - Target organ effects without systemic toxicity measures

Overall clarity in the decision process for selecting endpoints would provide transparency.

Preliminary Evidence Tables (ETBE/TBA)

Selection of Studies and Endpoints for Data Extraction

Request further explanation for not extracting data from the following studies;

- Dermal exposure studies,
- Acute or short-term exposures (less than 90 days); except in the context of immunotoxicity, neurotoxicity, developmental or reproductive toxicity, and
- Endpoints related to possible mechanisms of toxicity

Preliminary Exposure Arrays (ETBE/TBA)

- The presentation of results in the exposure response arrays is questionable; ***Statistical findings alone may not be meaningful and often severity of changes should be considered.***
- Recommend consistent presentation of lesions; ***Easier to compare across studies.***
- In the ETBE review; hepatocellular adenomas (benign neoplasms) have been combined with hepatocellular carcinomas (malignant neoplasms). ***Recommendation: presentation of malignant neoplasms should be separated from benign neoplasms with pre-neoplastic lesions having a separate category.***
- There were many related endpoints not mentioned in the tables/figures for the very comprehensive DART studies. ***Recommendation: Clarify criteria for endpoint selection for inclusion in summary tables and arrays. A narrative for each subsection would be useful.***

ETBE: Non-Cancer Endpoints

Preliminary Evidence Tables and Exposure-Response Arrays

Kidney Effects – Tables and Figures 2.1 and 2.2

Liver Effects - Tables and Figures 2.3 and 2.4

Reproductive Effects – Tables and Figures 2.5

Body Weight Effects – Tables and Figures 2.6 and 2.7

Other Systemic Effects- Tables and Figures 2.8 and 2.9

ETBE: Kidney Effects (Tables 2.1 and 2.2)

- Consistency in citing primary references (Suzuki et al., 2012 vs. JPEC, 2010a); ***Atypical hyperplasia, papillary necrosis, average severity of CPN, and relative kidney weights were extracted from JPEC report and not Suzuki publication***.
- Recommend removing category for kidney weights from 2-year study; ***Questionable endpoint to evaluate toxicity at the end of a chronic study.***
- Recommend removing category for *incidence of hyaline droplets* from 2-year study; ***Suggests a lack of understanding of the biology underlying α2u-globulin nephropathy.***
- Fully utilize the depth/amount of information in Cohen et al., 2011 study; ***Currently restricted to data on nephropathy (CPN).***
- EPA states that both quantitative and qualitative data is to be considered; ***Recommend Including granular casts, a lesion characteristic of α2u-globulin (α2u-g) nephropathy.***

ETBE: Kidney Effects (Tables 2.1 and 2.2)

- Recommend removing category of urinalysis endpoints (JPEC, 2008c; JPEC, 2010a; Suzuki el al., 2012); ***Casts in the urine and granular casts at the junction of the outer and inner stripes of outer medulla of kidney are totally different entities with different biological implications***
- Corrections in Table based on review of study report (JPEC, 2010b)
  - EPA states that mineralization in the papilla was *not examined* in female rats, it should be changed to *not observed*; ***mineralization in the papilla was observed in male rats only, implies females were examined but the lesion was absent.***
  - EPA states that atypical tubule hyperplasia was *not examined*; ***should be stated as not observed.***
  - Hyperplasia of the papilla observed in this study was not included; ***Papillary hyperplasia is a lesion that results from, and accompanies advanced CPN, clarifying that CPN is unique from chronic nephropathy.***

ETBE: Kidney Effects (Tables 2.1 and 2.2)

Recommend reviewing the following publication for inclusion:

Hard G.C., Banton M.I., Bretzlaff R.S., Dekant W., Fowles J.R., Mallett A.K., McGregor D.B, Roberts K.M., Sielken Jr. R.L., Valdez-Flores C., and Cohen S.M. (2013). Consideration of Rat Chronic Progressive Nephropathy (CPN) in Regulatory Evaluations for Carcinogenicity. *Toxicological Sciences*. 132(2):268-275.

Although there is no quantitative data in this paper, it describes the link between CPN and induction of renal tubule tumors.

ETBE: Liver Effects (Tables and Figures 2.3 and 2.4)

- Recommend including studies of shorter duration (< 90-days); ***Early changes can be used to interpret longer term responses (i.e. liver enzyme induction and histopathology).***
- Remove categories evaluated at the end of 2-year studies; ***Liver weight changes, clinical chemistry, and centrilobular hypertrophy.***
  - Sellers et. al., 2007 , "In these long-term studies, normal physiological aging changes and inter-current disease may contribute to inter-animal variability, which will confound organ weight interpretation (Long et al.,1998a; 1998b).
  - The Society of Toxicologic Pathology recommends organ-to-body weight ratios in toxicology studies ranging from 7 days to 1 year.
  - Liver enlargement in response to hepatic enzyme induction is typically associated with centrilobular hypertrophy (Maronpot et al., 2010), not typically observed following 104 weeks (Sellers et al., 2007).

ETBE: Liver Effects (Tables and Figures 2.3 and 2.4)

- Provide rationale for inclusion and exclusion of specific liver toxicity endpoints; ***Clinical chemistry: ALT vs. cholesterol***.
- Consistency in citing primary references; ***Unclear as to where study data is extracted (study reports vs. published papers).***
- Clarify for how data was extracted, transformed, and statistically analyzed for evidence tables.
- Recommend that quality control checks are conducted on data presented in evidence tables prior to review; ***Specific errors are identified in Lyondell's written comments.***
- Recommend that a distinction is made between adaptive vs. adverse effects; ***Liver weight increases accompanied by hepatocellular hypertrophy are common events in toxicity studies in rodents.***

ETBE: Liver Effects (Tables and Figures 2.3 and 2.4)

- Clarify why liver weights are only extracted from F0 generation; ***Gaoua, 2004b; Fujii et al., 2010; JPEC, 2008e.***
- Insure that data extracted is not misrepresented; ***Centrilobular hypertrophy was not evaluated in all the animals (Gaoua, 2004b).***
- The exposure-response arrays of liver effects was duplicated; ***Figure 2.4, Exposure to ETBE via inhalation is a copy of Figure 2.3, ETBE oral administration.***
- Recommend inclusion of additional endpoints; ***livers of mice exhibited time- and concentration-dependent increases in hepatocyte labeling index, quantitative data for response array and mode-of-action (Medinsky et al., 1999; Bond et al., 1996a).***

ETBE: Reproductive and Developmental Effects

CA OEHHA DART (Nov. 21, 2013) Committee voted unanimously that ETBE has **not** been clearly shown to cause developmental toxicity, male reproductive toxicity, or female reproductive toxicity.

Therefore ETBE will be removed from the Proposition 65 list.

ETBE: Reproductive Effects (Tables and Figures 2.5

- Provide clarity on data extracted and transformed; ***Include information on sample size based on litter and unit of analysis (litter vs. individual pup).***
- Confirm that sample size reflects litters assigned to exposure groups and not number of impregnated dams; ***Litter of origin is the appropriate experimental unit of analysis.***
- Recommend quality control checks on evidence tables prior to review; ***Specific errors are identified in Lyondell's written comments.***
- Consistency in citing primary references; ***Unclear as to where study data was extracted, F1 post implantation and total litter losses, (Fuji et al.,2010 or JPEC, 2008e).***

ETBE: Reproductive Effects - Tables and Figures 2.5

- Provide criteria for endpoint selection; ***Suggest inclusion of more explanatory text for selecting specific endpoints (i.e. Berger and Horner, 2003).***
- Caution with extracting data from reports translated to English; ***Recommend a complete understanding of how data is collected (Fujii et al., 2010 ; JPEC, 2008e).***
- Clarification of post-implantation loss; ***Reported differently in the original publications vs. in the evidence table (Fujii et al., 2010 ; JPEC, 2008e).***
- All data extracted into Evidence Tables should be checked for consistency and accuracy; ***(Gaoua, 2008; Asano et al., 2011; JPEC, 2008j).***

ETBE: Body Weight- Table and Figures 2.6 and 2.7

- Consistency in citing primary references; ***Unclear from what report the body weight data was extracted (Hagiwara et al., 2011 vs. JPEC, 2008d)***
- Request clarity as to why EPA only evaluated body weights for F0, and not the F1 generation; ***(Gaoua, 2004b).***
- Recommend that body weight assessments consider (female) reproductive stages (i.e., mating, pregnancy, lactation) as well as life stages (pups, adults); ***a single body weight value does not representative all possible changes in this study (Fujii et al., 2010).***
- Recommend that body weight changes incorporate changes in feed consumption and water intake to provide more meaningful data.

ETBE: Other Systemic Effects
Tables and Figures 2.8 and 2.9

- Request explanation as to why responses were combined on one exposure array; ***antibody response, immunological effects, adrenal weight, and mortality.***
- Consistency in citing primary references; ***spleen and adrenal weights (Suzuki et al., 2012 vs. JPEC, 2010a).***
- Insure data from reports is represented correctly; ***Evidence table notes a statistically significant change in relative adrenal weights for male rats whereas the report does not (JPEC, 2010a).***
- Include relative adrenal and spleen weights in evidence tables; ***provided in publication the following reports/publications, Medinsky et al., 1999; Bond et al., 1996.***
- Spleen and adrenal weights only presented for F0 and not F1 generation; ***(Gaoua, 2004b).***

ETBE: Other Systemic Effects
Tables and Figures 2.8 and 2.9

- Rationale for inclusion and exclusion of data; ***Wild-type and knock-out mice evaluated, data presented for only knock-out mice only (*Weng et al., 2011).**
- Recommend describing how numerical data is extracted from publication figures; ***Numerical percent change reported in Evidence Table (*Weng et al., 2011).**
- Recommend evaluating study quality prior to inclusion of data; ***Study quality concerns including design and methods (Weng et al., 2011).***
- Rationale for inclusion and exclusion of specific data; (Li et al., 2011)
  - Only data on one of two strains was included
  - Not all hematological and immunological markers were included
  - Extraction numerical data from publication figures

Additional Studies/Reports - ETBE

- JPEC, 2008h/Aso et al., 2013; Prenatal Developmental toxicity study in rats.
- Kakehasi et al., 2013; Mode of action of ethyl tertiary-buty ether hepatotumoigenicity in the rat: Evidence for a role of oxidative stess via activation of CAR, PXR and PPAR signaling pathways. Toxicol. Applied Pharmacol. 273 (2013) 390-400.
- Gaoua, 2003, Male rat pilot study
- Malarkey, 2011 Summary Report sponsored by the NTP and EPA of Pathology Materials from Selected Ramazzini Institute Rodent Cancer Bioassays, November 29, 2011.

TBA: Non-Cancer Endpoints

Preliminary Evidence Tables and Exposure-Response Arrays

- Kidney
- Thyroid
- Reproductive
- Development
- Neurodevelopmental
- CNS
- Body weight
- Liver
- Urinary Bladder

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CHECK TABLE AND FIGURE NUMBERS

TBA: Kidney Effects

- Insure study results are clearly represented; ***It is stated "There were no changes in kidney histopathology observed" implies that the kidneys were evaluated. Kidneys were fixed, but not microscopically examined. Lyondell Chemical Company, 2004 -should be cited as HLS, 2004.***
- Study quality evaluated; ***Histopathologic observations not diagnosed with accepted terminology (Acharya et al.,1995, 1997).***
- Qualification of lesions; ***It should be noted that mineralization was mainly at the "corticomedullary" junction. This lesion represents an imbalance of the Ca/PO4 ratio and is affected by dietary factors, and not necessarily an indication of nephrotoxicity. It is distinct from linear mineralization seen in the papilla in the 2-year study, which is a "marker" of a preceding α2u-globulin nephropathy event the (NTP, 1995; Cirvello et al., 1995).***

TBA: Kidney Effects

- Recommend inclusion of key endpoint; ***Hyaline droplet accumulation is an important pathologic entity that increases in all TBA exposed male rats.***
- Clarify conflicting text within the preliminary materials; ***It was stated that "no renal histopathological changes reported in mice at 13-weeks" and also stated that "histopathologic data for the 13-week study were not provided."***
- Recommend clarifying histological lesion with a footnote; ***On re-examination of kidney slides from the 15-month time point (NTP, 1995), it was determined that the mineralization in male rats was linear mineralization in the papilla, confirming a chronic end-point associated with α2u-g nephropathy occurs at an earlier time point (Hard, 2005).***

TBA: Kidney Effects

- Recommend clarifying histological lesions with a footnote; ***For both males and females, transitional epithelium hyperplasia and suppurative inflammation were identified by NTP as being part of the nephropathy (CPN) complex.***
- Extracted data from these publications should be included in the Histopathology subsection; ***Takahashi et al., 1993; Williams and Borghoff, 2001; Hard et al., 2001.***

TBA: Thyroid Effects

- Clarify that thyroid weight changes were not evaluated; ***It would be useful to list this endpoint and note that weight changes were not evaluated in the 13-week or at the 15-month interim necropsy.***
- Clarify the reasoning for the exclusion of selected thyroid histological changes; ***Besides follicular cell hyperplasia, other histological changes were noted but not included in the evidence table (C-cell hyperplasia, follicle, cyst, ect).***
- Clarify the timing of when follicular cell hyperplasia was observed in evidence table; ***Observed following chronic exposure to TBA (2-year study).***
- Clarify the lack of data provided in the evidence tables at the high TBA exposure concentration***; Lethality at this exposure concentration should be noted.***

TBA: Reproductive, Developmental, and Neurodevelopmental Effects

- Clarify the reasoning for including single acute exposure study; ***A single acute exposure of TBA in male mice at dose levels(up to 2,000 mg/kg with endpoints measured several days later (Billitti, et al., 2005).***
- Clarify criteria for data inclusion and exclusion from the following reports;
  - Lyondell Chemical Company (2004) OECD 421
  - NTP (1995) B6C3F1 mouse study
  - NTP (1997) F344/N rat inhalation rat study
  - NTP (1997) B6C3F1 mouse inhalation study

TBA: Reproductive Effects

- An MTBE rat inhalation study (Bevan et al.,1997) is currently included in the "Excluded (not pertinent)" category of the Reference List; ***MTBE is metabolized to TBA which is the primary metabolite found in rats following exposure. Since systemic blood levels of a chemical are required to cause reproductive toxicity, inclusion of the MTBE two-generation study is well-justified within the TBA IRIS process.***
- Reconsider inclusion of the Grant and Samson, 1982 paper based on quality; ***poorly reported and experimentally limited study in which half of the pups died due to experimental procedures that were very stressful (e.g. cannulation of pups raised away from their dams in a cup).***

TBA: Developmental Effects

- Incomplete and/or exclusion of endpoints without clear rationale; ***the Lyondell Chemical Company (2004) OECD 421***
- Questionable study quality and/or lacking details for inclusion.
  - Daniel and Evans (1982).
  - Faulkner, et al., (1989) C57BL/6J mouse study is incomplete.
  - Nelson et al., 1989

TBA: Neurotoxicity and CNS Effects

- Recommend avoiding using catch-all classification of "neurodevelopmental"; ***general categories such as neurobehavioral (cognitive, motor), neuropharmacologic, neuropathologic, or neurophysiologic findings should be used to avoid conflicting results.***
- The selection process for including significant or non-significant findings is not transparent; ***Although cited as a source of data, none of the findings from Nelson et al. 1991 paper were included in the exposure array.***
- The following citations should be re-reviewed to determine quality of the publication and then recommend including complete data sets with complete data entry.
  - Grant and Sampson, 1981
  - Wood and Laverty, 1979
  - Snell and Harris, 1980
  - Thurman et al., 1980
  - McComb and Goldstein, 1979a and 1979b

TBA: Body Weight

- Tables recording absolute and relative (body weight) liver weights; ***Consider brain weight as the basis for relative liver weights. It has been recognized that brain weight is more stable than body weight.***
- Although exposure (to females) occurred during pre-mating, mating, gestation, and lactation, the body weight data presented appears to be only the lactation body weights; ***HRL, 2004.***
- Numerous transcription errors were identified in the tables; ***NTP, 1995.***
- Body weight changes following inhalation exposure to TBA should have been included in the evidence table***; NTP, 1997.***

TBA: Liver Effects

- Not all liver effects reported in this paper were included in the table; ***Acharya et al., 1995.***
- Clarify the inclusion and exclusion of selected endpoints; ***in general only selected measurements of liver toxicity where included.***
- Figure 2-6; Errors identified in the exposure-response array of body weight, liver effects, and urinary bladder effects following oral exposure to TBA; ***Data reported in this array needs to be corrected or alternatively this figure could be deleted.***
- Recommend reviewing Blanck et al., 2010 for inclusion; ***Relevant endpoints for mode of action for hepatic enzyme inducer.***
- Recommend including reviews by McGregor et al., 2010; ***Provides a detailed evaluation of all endpoints discussed and should be considered in the overall evaluation.***

Summary Comments Pertaining to ETBE/TBA Preliminary Materials

- Preliminary materials are intended to provide opportunity for early discussion; ***however, content presented does not adequately support this objective.***
- Lack of consideration of study quality; ***A systematic approach for evaluating the quality of experimental data should be considered before accepted for any further evaluations (such as evidence tables). Recommended approach comparable to Klimisch scores. (Klimisch et al., 1997)***
- Time allotted for public review was limited; ***Encourage EPA to QC on data extracted and provide clear identification of where in report/publication the data was extracted from, allowing more transparency in the review.***

Summary Comments Pertaining to ETBE/TBA Preliminary Materials

- Preliminary materials do not adequately provide the documentation for a systematic review of the scientific literature.
- Approach used for creating evidence tables and exposure-responses arrays does not identify the findings that are meaningful and does not relate endpoints that need to be evaluated together (e.g. organ weight changes and body weight changes, toxicity and cancer endpoints).
- Inconsistent criteria for use of mechanistic data. EPA's application of these conflicting criteria is also inconsistent with some mechanistic information included, whereas other data is excluded. Recommendation: Data on key health endpoints much be included as pertinent to assessment of these endpoints.

These and our other comments identify significant deficiencies in the preliminary materials that EPA is encouraged to address before proceeding with preparation of the draft assessment.

In Summary

- The present preliminary materials, although a good start, at this point, do not satisfactorily achieve their purpose.
- There are significant deficiencies in the preliminary materials that EPA is encouraged to address before proceeding with preparation of the draft assessment.
- Overall, there is concern as to whether there will be another opportunity for the public to review the final "preliminary materials" once a completed and corrected data set are finalized and prior to the final IRIS assessment.